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Ecology of Ginger Rhizome Rot Development Caused by *Pythium myriotylum*

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*Pythium myriotyrum*에 의한 생강뿌리썩음병의 발생생태

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ABSTRACT : Lesion enlargement of ginger rhizome rot was most rapid at 35~40 C, but delayed greatly as temperature decreased. Time needed for killing a ginger plant, 22~25 cm long, was about 5 days at 35~40 C, but was 15 days at 15 C in a growth chamber test. Higher RH above 90%, higher soil moisture level above 80% of maximum soil moisture capacity, and deeper planting below 4cm enhanced the lesion development on ginger stems and rhizomes. *Pythium myriotylum* existed in field soil as forms of hyphal portion, hyphal swelling body, or oospore- or zoospore-like bodies, and served as the origin of its colonization. Inocula of *P. myriotylum* was randomly distributed in soil surface around ginger plants, but its density was decreased as increasing soil depth with the highest density at 0~10 cm soil depth. Population density of *P. myriotylum* did not vary significantly between the rhizoplane and the rhizosphere soil of a ginger plant, but differed greatly between the diseased and healthy plants with several to several hundreds times higher population in the diseased plants. A positive curvilinear relationship was found between *P. myriotylum* density and ginger rhizome rot severity.

***Key words*: ginger, *Zingiber officinale*, rhizome rot, *Pythium myriotyrum*, disease ecology, inoculum density, inoculum distribution.**

Rhizome rot of ginger has been a serious problem in major ginger producing areas in Korea (3) and all over the world (1, 9, 10, 11). Incidence of the disease averaged as 18.1% in Choongnam province in Korea in 1995 (3). It has been experienced by many ginger-growing farmers that the disease becomes severe and often devastates ginger fields, particularly in the year of extremely hot summer and many rainfalls in August and September.

Studies on ginger rhizome rot disease in Korea have been much concentrated on its etiology and screening of effective fungicides (4, 12). However, little research effort has been made on ecological aspect of the disease such as inoculum distribution in soil, and influence of soil environmental factors. In fact, lack of the information on ecology of ginger rhizome rot development has hampered establishment of effective control measures against this disease.

This study was carried out to examine effects of temperature, relative humidity, soil moisture, and planting depth on ginger rhizome rot development, to elucidate ecology of pathogen inoculum in soil including its distribution and survival forms, and to investigate the relationship between inoculum density and disease development. This study is a part of the researches which were initiated in 1995 to develop effective control measures against ginger rhizome rot disease. Research results on the survey of incidence (3), etiology (4) of the disease, and preliminary results (2) of the present study have been published.

MATERIALS AND METHODS

Isolates. The pathogenic *Pythium myriotylum* isolate 9-3 was used in the inoculation tests. Origin of the isolate has been described previously (4). The isolate

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was grown in potato dextrose agar (PDA) (5) at 35 C for 2 days and its culture disc was used as a point source of the inoculum in some experiments. The isolate was also grown in autoclaved artificial soil media (peat : vermiculite: perlite=3 : 1 : 1, v/v)) containing 1% corn meal for 2 weeks at 35 C and used as an inoculum in soil inoculation tests.

Ginger plants. Ginger cultivar Seosan-jaerae was grown in pots (50×40×10 cm) containing artificial soil (peat : vermiculite : perlite=3 : 1 : 1, v/v) in a greenhouse at 21~39 C. Healthy young ginger plants, approximately 22~25 cm long, were chosen and used as test plants in the inoculation experiments.

Examination of temperature and RH effects on disease development. To examine the effect of temperature on disease development, ginger plants were removed from the pots, washed thoroughly under tap water and put into polyethylene bags (30×35 cm), one in each, containing a wet paper towel to maintain moisture. The inoculation was achieved by placing a 0.5 cm diam. PDA culture disk of *Pythium myriotylum* on the crown of ginger plants. The polyethylene bags were sealed, and put in a multi-booth incubator set at 15 C to 40 C at 5 C interval with a 12 hr light/dark regime. In the RH experiment, ginger plants were washed, air-dried for 30 min, and put on a holder in plastic boxes (30×28×13 cm) containing different amount of sulfuric acid to obtain RH levels of 100, 96, 93, 89, 76, and 67% (5). The plants were inoculated similarly with a culture disc, and incubated at 30 C in a growth chamber with a 12 hr light/dark regime. In both experiments, rate of lesion enlargement on ginger stems and degree of rhizome decay were examined 4 and 11 days after inoculation, respectively. Each treatment has 3 replications (=plants) in both experiments.

Examination of soil moisture and planting depth effects on disease development. To examine soil moisture effect on disease development, ginger plants were transplanted into pots (12×15 cm) without any draining hole, containing the artificial soil media infested with *Pythium myriotylum*. Soil moisture levels in each pot were adjusted to 100%, 80%, 60%, 40% and 20% of maximum soil moisture capacity of the soil medium by managing volume of the irrigation water. Surface of the soil medium in each pot was covered with a polyethylene sheet to prevent excessive evaporation of the moisture.

In the planting depth study, ginger plants were transplanted at the depth of 2 cm, 4 cm, 6 cm, 8 cm, and 10 cm from the soil surface so that the upper part of crown of the plants was located at the designated depth in the infested soil medium in pots (12×15 cm). The pots were placed in the greenhouse. In both experiments, lesion length on ginger stems, number of leaves with yellowing, and degree of rotting of rhizomes of the plants were examined 11 days after transplanting (=inoculation). Each treatment was replicated 3 times in both experiments.

Enumeration of *P. myriotylum* in soil and identification of its survival form. In order to determine horizontal distribution of *P. myriotylum* in soil in ginger fields, 3 concentric rings, 5 cm, 10 cm and 15 cm in diameter, from a ginger plant, served as a center, in fields at Seosan area were drawn on the soil surface, and then 8 radial lines were made from the center towards north, south, west, east, northwest, northeast, southwest, and southeast. Soil samples, 5~10 g/spot were taken from the soil surface (0~5 cm depth) at each crossing spot of the 8 radial lines on three concentric rings to make a total of 25 soil samples including the center spot. Soil samples were made from 6 different locations randomly selected in a ginger field. To enumerate *P. myriotylum* density, 1 g soil sample was put on the selective medium (8) in a 9 cm diam. petri dish, added 10 m*l* of sterile water, and distributed the soil evenly on the surface of the medium. The dishes were incubated at 35 C for 18 hr, and then washed the soil off under a tab water. Number of colonies appeared on the medium surface was counted. To examine vertical distribution of *P. myriotylum* inocula in soil, soil samples were taken by a soil sampler from 5 locations randomly selected in a ginger field at Seosan area. Soil samples were thinned by the depth of 0~5 cm, 6~10 cm, 11~15 cm, and 16~20 cm. Density of *P. myriotylum* in the soil samples was examined similarly. Each soil sample was replicated 5 times for enumeration.

In order to examine survival forms of *P. myriotylum* in field soil, some of the colonies appeared on the selective media in the above experiments were examined under a microscope to observe origin of the colonies at very early stage of their colony formation. About 300 colonies were examined in the two separate experiments.

To examine the population density of *P. myriotylum* in rhizosphere and rhizoplane of the diseased and the healthy ginger plants, 3 plants were sampled each from two ginger fields at Seosan area in mid-September.

Soil closely cringed to rhizome surface collected by a gentle brushing, after removing soil lumps off, was used as a sample of rhizosphere soil. *P. myriotylum* density in the collected soil was examined on the selective media by the method described in the enumeration experiment. To examine rhizoplane population of *P. myriotylum*, an one cm^2 piece of the rhizome surface of the samples used in rhizosphere study was cut off, and washed in 10 m*l* of sterilized water by agitating it in the water. One m*l* of the water was dispensed on the selective medium. Number of colonies appeared on the selective medium was examined similarly.

Examination of the relationship between density of *P. myriotylum* and rhizome rot severity in fields. A 200~300 g of soil sample was taken from each of 15 different ginger fields with differing degree of rhizome rot severity in Seosan area in mid-September. Rhizome rot severity of each field was examined as % plant diseased. Density of *P. myriotylum* in each soil sample was examined by the method described above, and expressed as 0, 1~5, 6~10, 11~30, and 30< per gram soil sample. Rhizome rot severity in each field was plotted against soil density of *P. myriotylum* to examine the relationship between the two factors.

RESULTS

Effects of temperature and RH on disease development. Lesion enlargement on ginger stems was most rapid at 35~40 C, and reduced significantly as temperature decreased (Table 1). Lesion development at 35~40 C was about 3 times faster than that at 15 C. Time required for killing a whole plant inoculated was about 5 days at 35~40 C, but prolonged at lower temperatures, particularly at 20 C or below.

Table 1. Effect of temperature on rhizome rot development on ginger plants inoculated with *Pythium myriotylum* isolate 9-3 in a growth chamber

Temperature (°C)	Rate of lesion enlargement (mm/day)[a]	Days required for complete plant death
40	26.5±2.7	5.3
35	30.0±1.4	4.7
30	22.5±1.8	6.2
25	21.2±0.9	6.6
20	17.5±2.0	8.0
15	8.8±3.1	15.9

[a] Lesion enlargement rate and its standard deviation on a stem of a ginger plant after inoculation on the crown of rhizomes by placing a culture disc of *Pythium myriotylum* isolate 9-3.

Table 2. Effect of relative humidity (RH) on lesion growth on ginger plants by artificial inoculations with *Pythium myriotylum* isolate 9-3 at 30°C in a growth chamber

RH (%)	Lesion enlargement rate (mm/day)[a]	Degree of rhizome decay[b]
100	29.0±3.5	++
96	19.4±2.1	++
93	14.8±3.0	++
89	17.8±2.7	+
76	11.0±1.8	+
67	4.4±1.3	+

[a] Lesion growth rate on ginger stems from the point of inoculation and its standard deviation.
[b] ++ : severe, + : moderate.

Lesion growth was most rapid at RH 100%, but slowed down sharply below this level (Table 2). Lesion growth rate did not differ much between RH 96 to 89%, but was greatly reduced below RH 80%. Degree of rotting of rhizomes was severe at RH 93% or higher.

Effect of soil moisture and planting depth on disease development. Rhizome rot was developed most severely when the maximum soil moisture capacity (MSC) reached 80% or above (Table 3). Lesion enlargement rate was retarded significantly below these levels. Number of leaves with chlorosis and degree of rhizome decay tended to decrease as soil moisture levels became lower. Disease development was markedly inhibited when MSC was 20% or lower.

Table 3. Effect of soil moisture on rhizome rot development of ginger plants that were transplanted into pots containing infested soil with *Pythium myriotylum* 9-3 at the designated soil moisture levels in the greenhouse

Soil moisture (% MSC[a])	Lesion enlargement rate (mm/day)[b]	No. leaves with chlorosis/total leaves in 3 plants tested	Degree of rhizome decay[c]
100	18.8±1.4	18/20	++
80	20.0±2.7	15/18	++
60	13.8±2.1	13/21	++
40	13.8±1.2	12/18	+
20	1.2±0.3	7/19	-
Uninoculated	0	0	-

[a] MSC: Maximum soil moisture capacity.
[b] Lesion enlargement rate on stems from the point of inoculation and its standard deviation.
[c] ++ : severe, + : moderate, - : none.

Table 4. Effect of planting depth on rhizome rot development of ginger plants that were transplanted into pots containing infested soil with *Pythium myriotylum* isolate 9-3 in the greenhouse

Location of the crown of the plant	Lesion enlargement rate (mm/day)[b]	No. leaves with chlorosis/total leaves in 3 plants tested	Degree of rhizome decay[c]
Soil surface	0.4±0.1	0/18	−
−2 cm	8.4±1.1	3/18	+
−4 cm	15.0±2.4	6/18	++
−6 cm	14.0±2.1	6/18	++
−8 cm	19.0±1.7	8/18	++
−10 cm	24.0±2.8	8/18	++
Uninoculated	0	0/18	−

[a] Lesion enlargement rate on ginger stems and its standard deviation.
[b] ++ : severe, + : moderate, − :none.

Deeper planting resulted in more severe disease development (Table 4). Rate of lesion enlargement, number of leaves with chlorosis, and degree of rhizome decay tended to increase with increasing planting depth. Lesion growth rate for the planting depth of 2 cm was one third of that of 10 cm.

Horizontal and vertical distribution of *P. myriotylum* in soil. Density of *P. myriotylum* in the surface soil (0~5 cm deep) varied from 6.9 to 15.8 cfu/g soil, depending on the spots sampled (Table 5). The variation was not associated with locations of the soil sampled.

Density of *P. myriotylum* was the highest in the soil depth of 6~10 cm, followed by 0~5 cm, 11~15 cm, and 16~20 cm, although the variations within the same depth were large between the location sampled (Table 6). The soil density was decreased sharply below 10 cm, and thus *P. myriotylum* density at 16~20 cm averaged one fifth of that at 0~10 cm.

Table 6. Vertical distribution of *Pythium myriotylum* as indicated by the density of its population in field soil with respect to soil depth in Seosan area

Soil depth (cm)	No. cfu/g soil[a]					Average
	Spot I	Spot II	Spot III	Spot IV	Spot V	
0~5	12	8.5	10	14.5	7.5	10.5±2.8[b]
6~10	7	8.5	36	8.5	21	17.2±12.4
11~15	15	6	2	4.5	6.5	6.8±4.9
16~20	0.5	0	3.5	0	10	2.8±4.9

[a] Data based on field soil sampled from 5 spots randomly selected on Jun 17, 1996.
[b] Standard deviation.

Survival forms of *P. myriotylum* in soil. Survival forms of *P. myriotylum* inocula existed in soil as examined by origin of the colonies on the selective medium at the initial stage of its colonization were hyphal portion, hyphal swelling, and oospore- or zoospore-like structures (Table 7). In the soil sampled on Jun 25, one month before the epidemic started, about 75% of the colonies was originated from hyphal portions, 12% from oospore-like body, and 9% from hyphal swellings. About 4% of the colonies was originated from soil particles, from which origin of the colonies was impossible to be distinguished. However, in the soil samples of Jul 15, about 10 days before epidemic started, 69% of the colonies was originated from soil particles, 23% from hyphal fragments, and 4% each from oos-

Table 5. Horizontal distribution of *Pythium myriotylum* as indicated by the density of its population in field soil with respect to the direction and the distance from a ginger plant in Seosan area

Distance (cm)	No. cfu/g soil at the direction of[a]							
	N	NE	E	SE	S	SW	W	NW
5	6.2	10.8	6.6	5.8	7.3	7.0	9.6	6.9
10	6.2	6.4	10.6	8.0	9.2	11.4	9.1	12.4
15	13.1	8.0	12.1	9.4	8.5	10.7	15.8	14.5

[a] N : north, NE : northeast, E : east, SE : southeast, S : south, SW : southwest, W : west, NW : northwest. Data based on the average of six locations. The values are means of six replications. Both factor effects of the distance and the direction were insignificant in the analysis of variance of the data.

Table 7. Survival forms of *Pythium myriotylum* in field soil as examined by origin of the colonies appeared on the selective media in early stage of its colonization

Origin of the colony	Number of the origin (%)	
	Soil sampled on Jun 25	Soil sampled on Jul 15
Hyphal portion	229 (75%)	17 (6%)
Hyphal swelling	27 (9%)	52 (17%)
Oospore-like structure	37 (12%)	11 (4%)
Zoospore-like structure	0 (0%)	12 (4%)
Soil particle[a]	12 (4%)	209 (69%)
Total number of colonies observed	305 (100%)	301 (100%)

[a] Origin of the colonies was indistinguishable from the soil particles.

Table 8. Comparison of the density of *Pythium myriotylum* in rhizosphere and rhizoplane between the diseased and the healthy ginger plants sampled randomly in two different ginger fields in Seosan area

Soil source[a]	No. cfu/g soil			
	Diseased plant		Healthy plant	
	Rhizosphere	Rhizoplane	Rhizosphere	Rhizoplane
Field A I	108	104	15	22
II	7	3	1	1
III	118	120	2	8
Field B I	26	31	0	0
II	40	56	0	0.3
III	24		0	0

[a] Soil samples were obtained from three diseased and healthy plants in two different ginger fields on Sep 16, 1996.

pore- and zoospore-like bodies. *P. myriotylum* density in rhizosphere and rhizoplane of the diseased and the healthy ginger plants. Density of *P. myriotylum* ranged 0 to 120 cfu/g soil, depending on the plants sampled (Table 8). *P. myriotylum* density was similar between rhizosphere and rhizoplane within a diseased or a healthy plant sampled. However, its density both in rhizosphere and rhizoplane differed remarkably between the diseased and the healthy plants, and was several to several hundreds times higher in the diseased plants.

Relationships between *P. myriotylum* density and rhizome rot severity in fields. A positive curvilinear relationship was observed between *P. myriotylum* density and rhizome rot severity (Fig. 1). Rhizome rot severity tended to increase as increasing density of *P. myriotylum* in soil, even though the variations in disease severity within the same density level was large. However, rhizome rot severity did not vary greatly within *P. myriotylum* density levels of 1~30 cfu/g soil, but increased sharply above this density level.

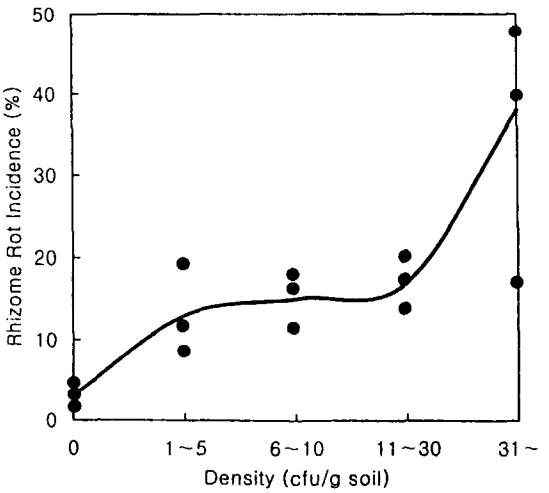

Fig. 1. Relationship between soil density of *Pythium myriolylum* and rhizome rot incidence when examined from 15 diffent ginger fields in Seosan area.

DISCUSSION

Ginger rhizome rot has been called as "heat disease" by farmers in major ginger producing areas. The name was given because the disease progresses rapidly, particularly in extremely hot summer days. The present study supports the farmer's observation, since optimum temperature for the disease development was found to be 35~40 C and plants at mid-growing stage were found to be killed rapidly at these temperatures within 5 days after infections. The daily maximum temperature of 35~40 C in Korea is observed usually in August after long rainy period which begins from late June to July. In ginger fields of major growing areas, the disease was developed most severely in this period. High temperature and high soil moisture conditions resulted from frequent rainfalls could play major roles for the severe incidence of the disease in this period. In Korea, daily summer temperature tends to increase year by year due to the global-based greenhouse effects. This might become another factor favorable for development of rhizome rot disease in the future.

Ginger rhizome rot has been recognized to be developed very severely when frequent rainfalls continue until late September after long rainy period. Severe disease development might result from the activation of zoospore inocula of *P. myriotylum* which disseminate through free soil moisture, and contribute to the late epidemic in September. This study also showed that the disease developed more severely when the maximum soil moisture capacity is 80% or above. Stimulation of the disease development by high soil moisture conditions observed in this study could explain partially the results of the survey conducted in 1995, where rhizome rot disease occurred more severely, especially at lower fields with poor drainage, lower sites of inclined ginger fields, drained paddy fields in which ginger plants were cultivated after rice, and fields with lower underground water level.

Ginger rhizomes are recommended to plant 3~4 cm deep in soil. The plants are growing usually in the soil depth of up to 10 cm. In this study, *P. myriotylum* inocula in soil was found to be rather randomly distributed around ginger plants in the depth of 0 to 10 cm, where the underground rhizomes grow. In this study the disease developed more severely as planting depth increases. This is thought to be due in part to that more areas of the ginger plants, such as emerging buds, basal stems, and crowns become exposed to soil inocula, when the rhizomes are planted more deeply.

In an experiment conducted in the naturally infested field in 1996 at Seosan area, rhizome rot disease started from many infection foci that scattered over the whole field, and eventually spread to adjacent areas to make large patches of dead ginger plants, until the epidemic devastated whole field. In a survey of 1995, a similar phenomenon was observed often in farmer's fields, where the disease occurred severely. The observations in the survey and results of the present study suggest that *Pythium myriotylum* inocula in soil may be randomly distributed around the ginger plants rather than in cluster, at least in severely infected fields.

Survival forms of the inocula in soil were found to be hyphal fragments, or oospore- or zoospore-like structures. This result is similar to the survival forms reported in Japan (6, 7).

In this study, large variations were found between the soil density of *P. myriotylum* and rhizome rot severity, although a positive curvilinear relationship was found between the two factors. The observed large variations in disease severity within the same level of the inoculum density appeared to be due in part to the differences between individual fields in cultural practices including cropping system, fertilization, water management, and disease control. Differences in soil properties and topography of the individual fields might also be a source of the variations. To reduce the variation, more number of fields are needed to include in the experiments.

Results of the present study could be used directly to reduce the incidence of ginger rhizome rot disease by means of cultural management practices, which result in lower soil temperature and soil moisture conditions, and shallow planting.

요 약

생강뿌리썩음병의 발병진전은 35~40 C에서 가장 빨랐으며 온도가 내려갈수록 병반진전속도도 감소하였다. 생육상 시험에서 초장 22~25 cm의 생강을 고사시키는데 걸리는 시간도 35~40 C의 고온에서는 5일이 소요되어 15 C의 15일에 비하여 1/3에 불과하였다. 상대습도 90% 이상의 고습도, 최대포장용수량의 80% 이상의 높은 토양수분, 재식깊이 4 cm 이상의 깊이심기는 뿌리썩음병의 병반진전속도를 증가시키는 요인이었다. *P. myriotylum*의 토양내 전염원은 균사절편, 팽윤균사편, 유사 난포자, 혹은 유주자의 형태로 존재하였다. *P. myriotylum*은 생강 주위의 토양표면에 임의로 분포하고 있었으며 표토로부터 10 cm 이내의 토양에서 가장 밀도가 높았고 그 이하 토양에서는 급격히 감소하였다. 한 생강식물의 근면과 근권토양내 *P. myriotylum*의 밀도는 큰 차이가 없었으나 건전주와 이병주 사이에는 큰 차이가 있어서 이병주에서의 밀도가 건전주에 비하여 수배-수백배 이상 높았다. 토양내 병원균 밀도와 뿌리썩음병의 발병정도와는 정의 곡선적 상관관계가 있었다.

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